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ORIGINAL RESEARCH

Neonatal, atopic and infectious disease outcomes among children born to mothers with latent tuberculosis infection

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Abstract: Exposure to microbes may result in maternal immune responses that can affect fetal immune development. Several lines of evidence have shown that mycobacterial antigens can change the onset of atopic disease. We hypothesized that infants born to mothers with a positive tuberculosis (TB) test and a negative chest radiograph, may exhibit differential development of atopic disease during early childhood. The study was designed as a case control study. Birth records for infants born to untreated mothers with a positive TB skin test (TST), as defined by ≥10 mm induration were reviewed (n = 145 cases) and compared to a randomly selected unmatched control cohort of 46 women with a negative TST who delivered during the same time period at Scripps Hospital in San Diego, CA, USA. Childhood outcome parameters reviewed were: (1) the onset of physician diagnosed asthma; (2) lower respiratory tract infection (LRTI) with wheezing, latent tuberculosis infection/wheezing diagnosed on physical examination; (3) nonsurgical hospitalization; (4) atopic disease (eye/skin/nasal-sinus disease); (5) infections: ear, LRTI, sinus. LRTI was defined as an infection of the lower airways, eg, pneumonia. Outcomes at the end of years 1, 2, and 3–5 years combined were analyzed. Fisher exact test, Chi-square analysis or Poisson regression analysis were used as appropriate and a *P*-value of <0.05 was defined as significant. The cases and controls had similar birth weights, gestational ages, maternal ages: 3.34 versus 3.35 kg; 38.3 versus 39.2 weeks, 27.4 versus 26 years (P = non-significant). The childhood outcome parameters of the new onset of asthma was significantly higher than controls by age 2 years, but not at other ages studied, based on available clinic follow up data ($P = 0.02$). There was a difference in the risk for lung infection at age 2 and 3–5 years ($P < 0.0001$). There were no differences in the other outcome parameters studied (P = ns). There were no cases of infants with a positive TST, maternal Bacille Calmette-Guerin vaccination or active maternal TB, based on our study findings. There was a higher occurrence of asthma and lung infections at age 2 years among controls ($P = 0.02$). Our study defines for the first time a possible influence of maternal latent TB infection on fetal and childhood illness.

Keywords: tuberculosis, fetal immune development, pediatric asthma

Introduction

The increasing occurrence of atopic diseases in modernized societies over the past few decades cannot be explained by genetic factors alone, but may reflect changes in the environment. Exposure to microbes may enhance the selection of Th1 versus Th2 phenotypes and produce immune modulatory responses which promote less active Th2 mediated inflammatory responses. Pediatric tuberculosis remains a global health problem. A few lines of evidence suggest that exposure to mycobacterial antigens changes the onset and course of atopic disease.[1,2] Immune development begins in utero and is a continuous process throughout childhood. Transplacental transfer of antigens,

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antibodies, cytokines, and cells can influence the maturation of the immune system.[3]

The concept of latent tuberculosis infection (LTBI, TB) is changing to include an ongoing spectrum of disease and immune responses. In LTBI there is a robust immune response involving both humoral and cellular immune responses. Peripheral blood monocytes for example, produce increased tumor necrosis factor (TNF) and interferon gamma in LTBI. The use of isoniazid (INH) which inhibits cell wall synthesis for 6–12 months may result in a decrease but not elimination of the risk of reactivation.

We hypothesized therefore that there may be less atopic disease among the infants born to mothers with a positive TB skin test (TST) (≥10 mm induration), and negative chest radiograph (cases) versus control infants.

Methods and study design

The study is a case control study with selected cases and random controls. The birth records of 2003–2005 from a busy tertiary care medical center in San Diego, CA, USA were reviewed and electronically searched for diagnostic codes related to maternal TB, or a positive maternal TST. A total of 145 cases of expectant mothers with a positive TST during routine prenatal screening, and a negative chest radiograph, without multiple drug TB treatment, defined as LTBI were identified. A positive TST was defined as ≥10 mm induration. A TB history and TST is included as part of routine prenatal visits in our population, and the mothers with TST positivity were part of a general population being seen for routine prenatal care.

A control cohort of 46 mothers with a negative TST, who delivered at the same hospital during this time period, and their offspring were included as randomly selected controls. The children in the control group were evaluated at the same neighborhood based clinics as the cohort group. Of note, in our population, the TST is a routine prenatal screening test regardless of symptoms.

Birth descriptive and outcome parameters

Birth weight, expressed in kilograms (kg), gestational age (GA) in weeks, Apgar score expressed as a score of 1–10 at one minute and 5 minutes after delivery, maternal age, maternal asthma history, method of delivery (non-C-section versus C-section), Group B streptococcus (GBS), neonatal intensive care unit stay, were compared between case and control infants.

Children who continued to be followed at the two major affiliated pediatric teaching outpatient sites were included for a chart review between birth–5 years. Physician visits for both well- and ill visits were reviewed. The cases included for further analysis were viable infants born at 36 weeks GA or more. Three infants were excluded for fetal demise, cardiac defects, and prematurity. There were no cases of congenital or childhood TB infections among the cohorts further reviewed for childhood outcome.

The specific outcome childhood parameters reviewed were: (1) The onset of physician diagnosed asthma; (2) lower respiratory tract infection with wheezing (LTRI/W) diagnosed by a physician; (3) physician diagnosed atopic eye, skin, sinus disease; (4) number of nonsurgical hospitalizations; (5) number of episodes of otitis media infections, lower respiratory infections (bronchitis/pneumonia), and sinus infections.

Familial and household environmental factors of: household smoke exposure, household pets, maternal asthma, number of siblings, and breast feeding duration (≥4 months) were noted. The development of maternal and childhood TB disease up to 5 years after delivery was recorded. The reported results 0–5 years were adjusted for the familial and environmental factors above.

Statistical analysis

Chi-square analysis and Fisher exact test were used as appropriate, and a P-value of <0.05 was considered significant. Generalized linear models were utilized to compare cumulative rates of atopic disease, and ear, lung, and sinus infections between the two cohorts over the 5 years of observation. We initially examined whether a Poisson regression model would provide an adequate fit to the observed infection counts, with years of exposure as an offset, and covariates of sex and group. For each type of infection, rates were significantly over-dispersed relative to a Poisson model (likelihood ratio test, $P < 0.001$ for each regression), so that we instead utilized negative binomial regressions for the comparisons. Calculations were performed in Stata 9 (StataCorp LP, College Station, TX, USA).

Results

Birth outcomes

The birth outcome analysis revealed that the case group (mothers with a positive TST), had more NICU (neonatal intensive care unit) admissions, (11 versus 2, $P = 0.53$). There were ten case versus two control infants with Apgar scores

less than 8 at one minute ($P = 0.077$). At 5 minutes, there were only three infants with Apgar scores < 8 (2 cases versus 1 control). Cases and controls were similar for these parameters: birth weight, 3.34 versus 3.35 kg; GA 38.8 versus 39.2 weeks; maternal age mean 27.4 versus 26 years ($P = 0.83$, 0.192, and 0.157 respectively), and delivery method. (C-section versus non-C-section) (P = non-significant [ns]). Maternal asthma was noted in five cases versus two control infants (P = ns). The frequency of GBS colonization was 21 versus 18.3% (P = ns). In the birth cohort there were 0 cases versus four control infants in smoking households ($P = 0.02$, Table 1). In performing the statistical analysis, this factor was controlled for accordingly in the results shown and did not change the outcome results.

Childhood outcomes

Physician diagnosed asthma, lower respiratory tract infection with wheezing and infections

The childhood outcome parameters of the new onset of asthma were significantly higher among controls at age 2 years, but not at the other ages studied, based on available clinic follow up data (P = ns). At the end of year two, 0 cases (n = 42) and four control (n = 25) subjects carried a diagnosis of asthma ($P = 0.02$). The occurrence of LRTI with wheezing, atopic skin, eye, or sinus disease, number of otitis media or sinus infections was not significantly different between the two groups. There were no statistically significant differences in nonsurgical hospitalizations years 0–5 (P = ns).

The control group, identified as having higher new onset of asthma at age two, also had a significantly higher number of lung infections at age 2 years ($P < 0.0001$). After adjustment for the potential modifying familial and household environmental factors listed in the methods section (eg, household pets, smoke, siblings), gender and delivery method, the results did not change. There were no cases of childhood TB, known maternal Bacille Calmette-Guerin vaccination, nor active maternal tuberculosis disease identified.

Table 1 The environmental and familial modifying factors among case versus control infants

Familial/household environment			
	Cases (n = 145)	**Controls (n = 46)**	***P*-value**
Maternal asthma	5	2	0.65
GBS + maternal	24	9	0.82
Smoke exposure	0	4	0.02*
Pets	4	0	0.58
Siblings (mean, n)	25	11	0.80

Notes: A *P*-value of <0.05 was considered significant; the statistical analysis adjusted for these factors.
* indicates statistical significance.
Abbreviation: GBS, Group B streptococcus.

Cumulative risk of infection

When considering lower respiratory tract infections, specifically the risk of lung infections in year 2 and year 3, there was a significantly lower number of lung infections among cases versus controls ($P < 0.0001$). In the analysis of other infections at years 1, 2, and 3–5 there were no significant differences (Table 2).

Overall by analyzing the years collectively in terms of total infections and rate ratios, in all years, there were a total of 130 ear infections, 90 lung infections, and 2 sinus infections in 103 subject-years in the case group (rates 1.26 per year, 0.87 per year, and 0.02 per year, respectively), and 90 ear infections, 150 lung infections, and 8 sinus infections in 68 subject-years in the control group (rates 1.32 per year, 2.21 per year, and 0.12 per year, respectively). Differences between the two cohorts did not achieve statistical significance when all 5 years were considered: the rate ratios (cases/controls) for ear infections, lung infections, and sinus infections were 0.73 (95% confidence interval [CI] 0.34 to 1.60), 0.38 (95% CI 0.22 to 0.68), and 0.17 (95% CI 0.0085 to 3.55) respectively.

Cumulative risk of atopic disease

There were 29 atopic events related to the eye, nasal passages, or skin, in 103 subject-years in the case group (rate 0.28 per year), and 32 atopic events in 68 subject-years (rate 0.47 per year) in the control group. Differences between the two cohorts did not achieve statistical significance: the rate ratio (cases/controls) was 0.61 (95% CI 0.24 to 1.54); Table 3.

Cumulative risk of any wheezing and asthma

When all years were considered and the groups compared, wheezing was reported in 9 of 104 subject-years in

Table 2 The cumulative infection rates among case versus control infants

Cumulative rate of infection: birth–3 years			
	Cases = 103 subject years	**Controls = 68 subject years**	***P*-value**
Ear	1.26/yr (n = 130)	1.32/yr (n = 90)	0.42
Lung	0.87/yr (n = 90)	2.21/yr (n = 150)	<0.001
Sinus	0.02/yr (n = 2)	0.12/yr (n = 8)	0.06

Note: A *P*-value of <0.05 was considered significant.

Table 3 The cumulative atopic rates among case versus control infants

Cumulative atopic events			
	Subject years	**Number of events**	**Rate/year**
Case group	103	29	0.28
Control group	68	32	0.47

Note: A *P*-value of <0.05 was considered significant.

the case group with an annual incidence rate of 0.0865, 95% confidence interval (0.039–0.164 per year) and in 0 of 67 subject-years in the control group with an observed annual incidence of 0, 95% confidence interval (0–0.055 per year), P = ns.

Weight gain during year one

The difference between birth weight and end of year one weight in kg was analyzed. At the end of year one, the mean weight difference among infants born to a mother with a positive TST was 6.47 kg ± 1.48 (n = 39). In comparison, the mean weight difference, at the end of year one, among controls was 6.93 kg ± 1.31 (n = 23). There was no statistically significant difference between the two groups. The cases and controls had similar weight gain year one (Table 4).

Maternal prophylactic treatment with INH

A few mothers (n = 23) were treated for LTBI with INH, prior to delivery. Most mothers were untreated for the duration of the pregnancy. We analyzed by two-sided Fisher's exact test, whether maternal INH treatment affected the childhood outcome 0–5 years, for total number of infections, LTRI with wheezing or development of atopic disease. In year 2, there were more atopic infants born to mothers with a positive TST who were treated, compared to infants of TST positive mothers who were not treated (P = 0.022). In all other years and outcome parameters there was no significant difference.

Discussion

Our study shows for the first time that infants born to mothers with latent TB infection, defined as having a positive TB skin test and negative chest radiograph, have similar gestational ages and birth weights, and 5-minute Apgar scores compared to control infants. In addition, we have shown for the first time that the childhood outcomes related to atopy and infection are generally similar, with an intriguing exception in year two. Our control cohort had higher rates of asthma and lung infections in year two compared to the cases. This finding did not extend over 5 years, but may represent transient protection against asthma and lung infections by skewing the immune profiles of these infants towards a Th1 or non-Th2 phenotype. The lower respiratory tract infection rate observed may in turn impact the development of asthma, if caused by certain viruses such as respiratory syncytial virus, which can induce prolonged bronchospasm.[4] Furthermore, maternal treatment for LTBI was associated with lower rates of atopic disease among case infants which lends further support to the immune modulatory effect of maternal LTBI on their infants. While this was a retrospective analysis, we recorded objective physician observed data, limiting the impact of subjective reports of wheezing or atopic symptoms which may mimic other diseases. In addition, we statistically adjusted for key environmental factors known to affect the rate of infection, atopic disease and asthma.

Our study was based on a hypothesis that in utero exposure to maternal *Mycobacterium tuberculosis* may result in transplacental transfer of Th1 cytokines such as Interferon (IFN)-gamma and therefore may alter the childhood outcomes of atopy and infection. Our study raises the possibility that in utero exposure to maternal immune mediated reactions to naturally acquired *M. tuberculosis* without actual TB disease may alter immune responses in early childhood. Our study adds new findings to the literature previously based on BCG (bacillus Calmette–Guérin) vaccination, animal studies and postnatal associations. Our study, however, is distinct in analyzing prenatal maternal factors related to naturally acquired TB infection, and utilizing physician diagnosis rather than parental surveys. While this is a retrospective analysis, we have taken several measures to limit study bias: (1) The hospital has standardized medical records and both maternal and infant records were searched electronically; (2) we used only provider based data rather than parental questionnaires; (3) because the providers were not aware of the study, their notes were not biased and included standard pediatric visit data per American Academy guidelines,[5] to which the two teaching sites adhered; (4) a detailed analysis of potential modifying factors was included based on extensive review of the literature; (5) lastly, among all the mothers, the TST was a routine prenatal evaluation, such that there was no selection bias.

Table 4 Weight gain year one: a comparison of case versus control infants

Weight gain year one			
Group	**N**	**Mean (gm)**	**Standard deviation**
Case	39	6474	1479
Control	23	6930	1311

Vaccination studies provide intriguing evidence that *Mycobacteria* are immunomodulatory. Vaccination with *Mycobacterium vaccae* ameliorates atopic dermatitis in one study of school age children 3 months after vaccination. The vaccination has been shown to alter expression of key cytokines such as interleukin (IL)-4, IL-5, interferon gamma, and transforming growth factor beta.[6] Another study of school children immunized with BCG vaccine showed a lower prevalence of atopic disease compared with non-vaccinated children in the same country.[7] One murine BCG study indicated a suppression of airway eosinophils and CD4+ regulatory cells in response to vaccination, which may help restore immune balance. BCG did not however demonstrate benefit to adult asthmatics receiving inhaled steroids in another study.[8] One possibility is that T regulatory cells or Tregs are suppressed by inhaled steroids and this confounded the results. Another study of neonatal mice receiving BCG concluded that there is a long term benefit in limiting eosinophilia and airway hyper reactivity. The authors highlight that these protective effects are in part due to the early modulatory effects of early rather than late exposure.[9] Vaccine responses though may have a different effect compared to natural infection. Among our maternal cohort, there were no patients with documentation of recent BCG to explain their TST results and the interpretation based on CDC guidelines infers naturally acquired infection among our adult, largely Hispanic, population. BCG infant vaccination is not part of routine US immunization protocols. In Mexico, the BCG is administered as part of early childhood vaccinations, but would not be expected to affect the TST interpretation in childbearing adults, since the reaction wanes over 5 years.

Epidemiologic evidence suggests that mycobacterial antigen exposure can modify immune responses. Based on World Health Organization databases worldwide the authors studied whether the lack of exposure to early childhood infections, including TB, may increase the risk of developing atopic disorders. The authors concluded that tuberculosis was significantly inversely related to the lifetime prevalence of asthma and wheezing.[10] This is the largest such study and involved over 200,000 children and 155 centers. Regional differences may arise from a questionnaire-based study (n = 5495) from Vietnam, with one of the world's highest rates of TB infection, which showed no inverse relationship to atopic disease.[11] A meta-analysis of 23 studies addressing this question, however, found cross-sectional analyses to support the inverse relationship between a positive TST and reduction in allergic symptoms (odds ratio = 0.63).[12]

Sharp et al studied peripheral cord blood mononuclear cells from children at risk for development of atopic disease, and found that these cells when stimulated with purified protein derivative produced higher levels of IL-5 and IL-10 at 6 months to 3 years in children who clinically developed atopic dermatitis.[13]

Our study focused on infants born to mothers with latent TB infection. Latent TB patients have too few bacteria to cause disease, but enough to induce specific immune reactions. The persistence of a positive TST despite treatment attests to the ongoing immune response to mycobacterial antigens. These reactions include specific Toll-like receptors and induction of antigen presenting cell activity. This in turn leads to cytokine and chemokine expression patterns and clonal expansion of Th1 cells which produce transferable cytokines such as IL-12 and IFN-gamma.[14] Patients with a positive TST, produce excessive serum cytokines and chemokines. Among these are predominantly, IFN gamma, monocyte chemoattractant protein, IL-15, and TNF-alpha.[15]

Previous studies of cord blood samples have shown that the prenatal and antenatal environment and cytokine production can influence the development of atopic disease in infancy.[16–18] In one study detectable IFN-gamma in cord blood, was associated with a lower physician diagnosis of asthma at age 6 years.[16] In another study of infants with severe bronchiolitis infection, higher levels of lipopolysaccharide-stimulated (LPS) cord blood mononuclear cell production of IL-6 and IL-8 were present compared to control infants.[17] Other studies[16–19] have demonstrated that there is decreased CD4+ T cell IFN-gamma production among infants who later developed atopic dermatitis (odds ratio = 5.16). Our study of infants born to mothers with latent TB infections coupled with the known associated production of IFN-gamma and other immunomodulatory cytokines, builds on the known literature in the study of cord blood, in utero fetoplacental cytokine production, and subsequent development of atopic disease.[16–19] Humoral responses in latent TB are not fully elucidated, but there is evidence that B cells in fact may modulate cytokine production.[20] Recently, a study of a single systemic administration of Ag85B of mycobacterial DNA inhibited allergic airway inflammation in a mouse model of asthma.[21]

Conclusion

In conclusion, our study shows for the first time, that infants born to mothers with latent TB infection may have transiently lower rates of lung infection and physician diagnosed asthma in year 2, than control infants. Infants born to mothers treated

for latent TB had a higher rate of atopic disease compared to infants born to untreated mothers. The birth outcomes, and year one weight gain was similar between both groups. The significance of this study is that we identified and analyzed a cohort of infants born to mothers who have a positive TST, compared with infants born to mothers with a negative TST. This study adds to current knowledge as to whether infants born to mothers with known reaction to *Mycobacteria tuberculosis* antigens are at any altered risk for development of subsequent complications. In utero, fetal immune responses possibly may differentiate in response to maternal TB specific immune responses. Early exposures to Th1 and Th2 cytokines during fetal development and infancy influence the development of the infant immune system. Future studies of the perinatal influence of latent TB on the development of atopic disease are indicated, and may provide insight on the role of fetal immune development in the pathogenesis of infant atopic disease.

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Disclosure

The authors have no conflicts of interest to disclose in this study.

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